A Multiwavelength Microflow Cytometer

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Introduction: Fluorescence-based flow cytometry dates back to the 1960s. Essentially, cells or particles are aligned in a flow stream and optically interrogated. Size, density, and fluorescence at multiple wavelengths can be quantified. In many cases, tags, such as fluorescently labeled antibodies, are mixed with the samples prior to analysis so that specific targets or cell functions can be identified. Currently, large, complex, laboratory-based flow cytometers are required to perform medical diagnostics, such as white blood cell counts and immunoassays to detect infection, or for environmental monitoring applications, such as classification of marine algae.

In the traditional design, the sample containing the particles or cells is pumped out of a small tube into a much larger, concentric pipe that is carrying filtered water. This hydrodynamic focusing puts all the particles into the center of the wider "sheath" stream, which then is tapered to a smaller diameter. The particles are thus "focused" and pass single-file through the laser beams for analysis. Over the last decade, flow cytometers have become smaller in size and less expensive, but this sheath flow design is not amenable to miniaturization to the point that the systems are portable. NRL's Center for Bio/Molecular Science and Engineering has developed a microfluidic sheath flow system that is robust, simple to fabricate, and very compact. This sheath flow device forms the basis of a microflow cytometer that has demonstrated the capability for 4-color analysis that is competitive with the larger, commercial systems.

The NRL Microflow Cytometer: Figure 7(a) shows a diagram of the microflow cytometer. The sample stream is shown in red. A picture of an actual chip in action can be seen in Fig. 7(d). The sample stream enters from a small middle channel and is flanked on either side by two sheath streams. These sheath streams are usually just water or a clean buffer solution. Because the flow rates of the sheath streams are much faster than the sample stream, the sample stream is compressed between them. Figure 7(b) shows a computer model of the cross section immediately after the streams are brought together. The sample stream is separated from the walls, but it still touches the ceiling and floor of the channel. This problem is eliminated as the liquid flows through the part of the channel with the chevron-shaped grooves in the ceiling and floor of the channel. As the fluid flows through the grooved region, the chevron grooves move fluid from the sides to the top and bottom of the channel, squeezing the sample stream vertically. The result is that the sheath stream completely wraps around the sample stream, yielding the cross-section shown in Fig. 7(c).

Once the sample is flowing in a narrow, confined stream surrounded by sheath fluid, it can be evaluated using the optical fibers shown in Fig. 7(e). The light from two lasers is launched into the channel using optical fibers. Two fibers, directly opposite the two excitation fibers, are used to carry the excess light away so that it will not interfere with the measurement. Two more fibers are used to collect the light that has interacted with cells or particles as they pass through the interrogation region. Some of the collected light is simply laser light that has bounced off the particles; this light scatter signal is proportional to particle size and density. Laser light which is absorbed by fluorophores either attached to or in the cells or particles is emitted at longer wavelengths. Filters at the other end of the fibers discriminate the colors prior to quantitation of the fluorescence using photomultiplier tubes.

Multiplexed Detection Assays: The microflow cytometer detects and distinguishes particles coded with two fluorescent dyes. These coded particles are coated with antibodies that bind specific targets such as bacteria or toxins. After exposure to the sample to be tested, tracer antibodies carrying a dye that fluoresces at a third wavelength bind to any target captured on the coded particles. The microflow cytometer measures the levels of the third fluorescent dye to detect and quantify the amount of the target present in the sample (Fig. 8).

With the ability to distinguish the multiple coded particles and detect the target analyte on each particle, the microflow cytometer is capable of detecting multiple targets simultaneously. Our initial 6-plex assay demonstrated limits of detection highly comparable to those obtained using a commercial laboratory system with the same (killed) targets, antibodies and coded particles. We obtained the following detection limits: 103 cells/ml for *E. coli*, 104 cells/ml for *Listeria*, 105 cells/ml for *Salmonella*, 1.6 ng/ml for choleratoxin, 0.3 ng/ml for Staphylococcal enterotoxin B, and 8 ng/ml for ricin.

Conclusion: This microflow cytometer system has demonstrated the analytical capability for applications in miniaturized, automated, point-of-care instrumentation for hospitals and portable field-deployable sensor systems for medical diagnostics, environmental monitoring, and biodefense.

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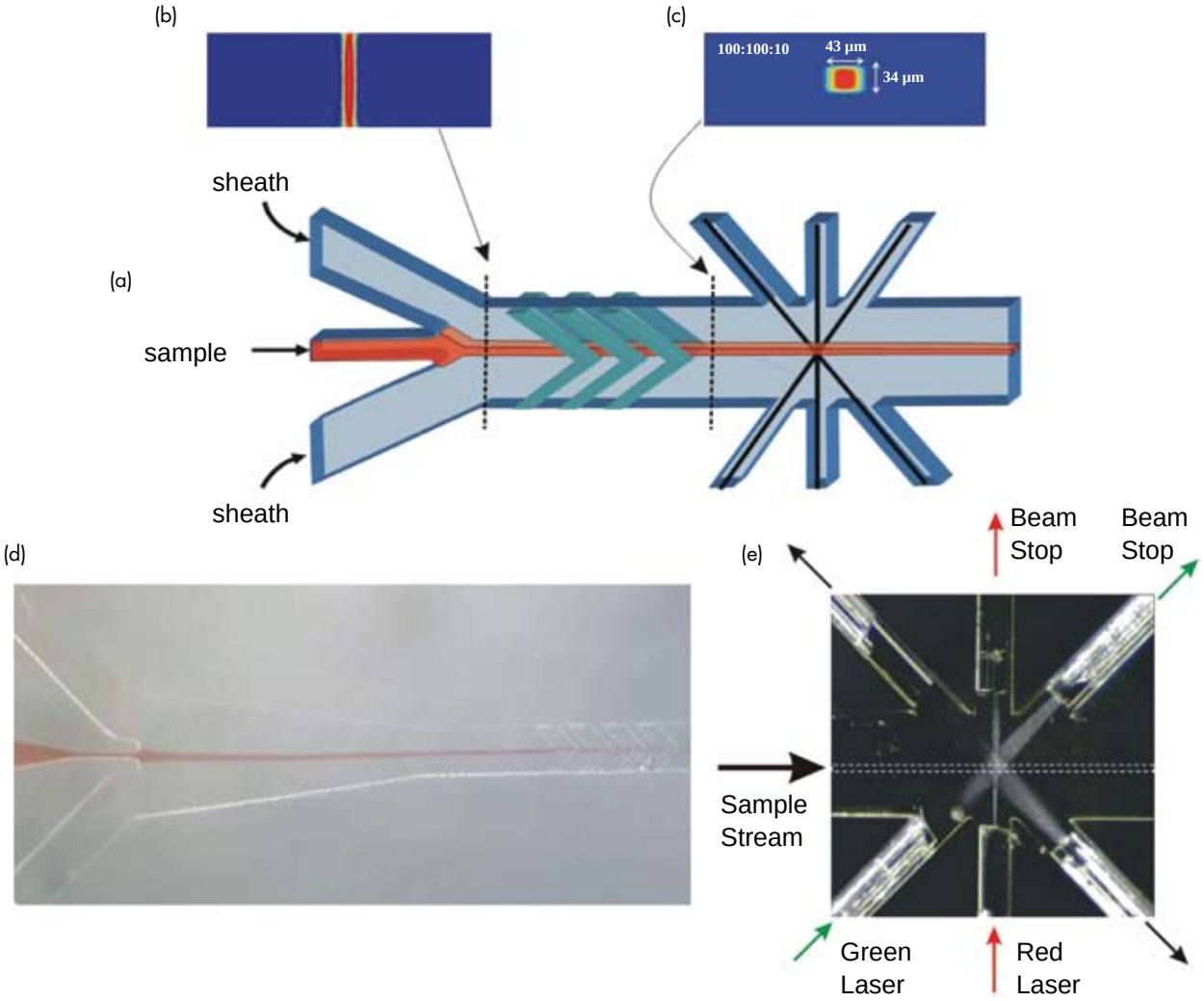

FIGURE 7
(a) diagram of microflow cytometer channel showing sheath and sample inlets and interrogation region, (b) cross-section of flow channel before chevrons, (c) cross-section of flow channel after chevrons showing sheath flow, (d) photograph of core stream with dye, (e) photograph of optical fibers illustrating the focusing of the numerical apertures of a single-mode excitation fiber and two multi-mode collection fibers on the interrogation region.

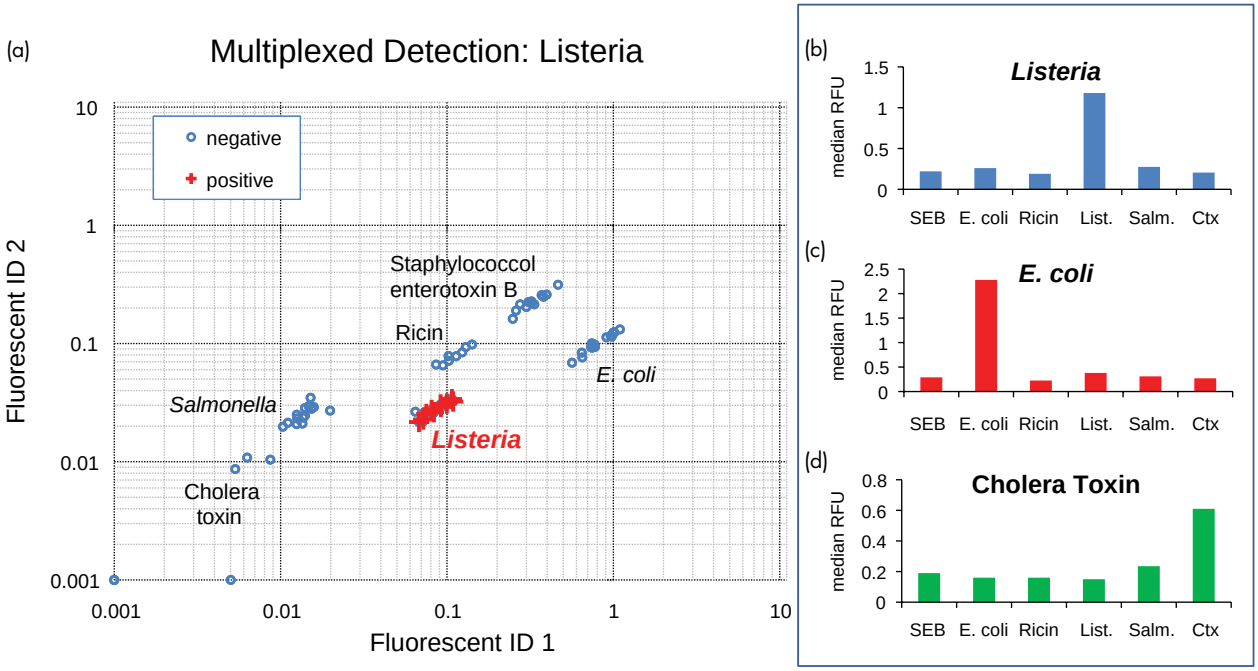

FIGURE 8
The microflow cytometer is capable of detecting multiple biothreat targets using its ability to distinguish fluorescently coded particles, each of which can be specific for a different target. By measuring the amount of fluorescent light, measured in relative fluorescence units (RFU) at two different wavelengths, the particles are identified, while fluorescence at a third wavelength is used to determine the presence or absence of each target. (a) shows the plot of the different particle sets identified based on the amount of two different fluorophores embedded in each particle. The presence of *Listeria* in this assay is indicated by the red marks on each particle that exhibited fluorescence at a third wavelength. The bar graphs on the right quantify the fluorescence at the third wavelength in particle sets to which different targets were added: (b) *Listeria* (10^7 cfu/ml), (c) *E. coli* (10^7 cfu/ml), and (d) cholera toxin (1000 ng/ml).